Colilert Testing

Procedures outlined in this section of the Lab Manual are taken from Standard Methods for the Examination of Water and Wastewater, 18th Edition (1992).

Colilert Test

Colilert Reagent is used for the simultaneous detection and confirmation of total coliforms and *E. coli* in water. Colilert utilizes nutrient indicators that produce color and/or fluorescence when metabolized by total coliforms and *E. coli*. When the reagent is added to the sample and incubated, it can detect these bacteria at 1 CFU/100ml within 24 hours with as many as 2 million heterotrophic bacteria/100ml present. The presence/absence test is used for drinking water samples and the quanti-tray enumeration procedure is used for raw water samples.

Presence/Absence Test Procedure

- Carefully separate one Snap Pack from the strip taking care not to accidentally open adjacent pack.
- Tap the Snap Pack to ensure that all of the Colilert powder is in the bottom part of the pack.
- Open one pack by snapping back the top at the scoreline.
- Add the reagent to the water sample in a sterile, transparent, non-fluorescent vessel.
- Aseptically cap and seal the vessel.
- Shake until dissolved.
- Incubate for 24 hours at 35°C ±0.5°C.
- Read the results at 24 hours. Compare each results against the comparator dispensed into an identical vessel.
  - If no yellow color is observed, the test is negative. Check vessel for fluorescence by placing a 6 watt 365nm UV light within 5 inches of the sample in a dark environment. If fluorescent, indicates *shigella*-type organisms.
  - If the sample has a yellow color equal to or greater than the comparator, the presence of total coliforms is confirmed. If color is not uniform, mix by inversion then recheck.
  - If the sample is yellow, but lighter than the comparator, it may be incubated an additional 4 hours (but no more than 28 hours total). If the sample is coliform positive, the color will intensify. If it does not intensify, the sample is negative.
  - If yellow is observed, check vessel for fluorescence by placing a 6 watt 365 nm UV light within 5 inches of the sample in a dark environment. Be sure the light is facing away from your eyes and toward the vessel. If fluorescence is greater or equal to the fluorescence of the comparator, the presence of *E. coli* is confirmed.

Quanti-Tray Enumeration Procedure for 100ml Sample

- Pour the sample reagent mix from step 6 above directly into the tray avoiding contact with the foil tab, and then seal the tray according to Quanti-Tray instructions.
- Incubate for 24 hours at 35°C ±0.5°C.
- Follow the same interpretation directions from step 8 above to count the number of positive wells. Refer to the MPN table provided with the Quanti-Tray to determine the Most Probable Number (MPN) of total coliforms (yellow wells) and E. coli (fluorescent wells) in the sample. The color and fluorescence of positive wells may vary.

Procedural Notes

- If an inoculated Colilert sample is inadvertently incubated over 28 hours, the following guidelines apply: Lack of yellow is a VALID NEGATIVE TEST. A yellow color after 28 hours is not a valid and should be repeated or verified.
- Some water samples containing humic materials may have an innate color. If a water sample has some background color, compare inoculated Colilert sample to a control blank of the same water sample.
- Use sterile water, not buffered water for making dilutions. Colilert is already buffered. Always add Colilert to the proper volume of diluted sample after making dilutions.
- Colilert is a primary water test. Colilert performance characteristics do not apply to samples altered by any pre-enrichment or concentration.

Quality Control Procedure

Quality control should be conducted on each lot of Colilert, or more often as regulations require. Inoculate sterile water (100ml) with the Quanti-Cult or American Type Culture Collection (ATCC) bacteria listed below. Follow the above test procedure, and compare test results to the expected results below.

Quanti-Cult Organism	ATCC #	Expected Result
E. coli	25922 or 11775	yellow, fluorescent
Klebsiella pneumoniae	31488	yellow, not fluorescent
Pseudomonas aeruginosa	10145 or 27853	clear, not fluorescent

#____AUTOCLAVE RUN SHEET

DATE	ITEMS STERILIZED	STERIL TIME	TIME		ELAP TIME	MAX TEMP	MAX PRES	QC CHECKS				TEC H
			IN	OUT				TAPE	CLOCK	SPORE +/-	SPORE REF #	

COLILERT LAB SHEET

Date:_____

Lab #	Sampler	Sample ID	Time	Cl_2	Type	Received Date	Set Up Time	Quanti-tray				P/A	Report Date	Report Time	Analyst
								# Yellow Wells	Total MPN	# Fluor. Wells	E. coli MPN				

Reviewed:_____ Date:_____ Entered:_____

INVENTORY LOG

REF #	DATE REC	ITEM	QUANT	LOT #	MAN. EXP DATE	OPEN DATE	OPEN EXP DATE	DISPOSAL	
								DATE	AMOUNT

SEMIANNUAL TEMPERATURE RECORD

INCUBATOR_____ WATERBATH_____ REFRIGERATOR_____

CERTIFIED THERMOMETER_____ INCUBATOR THERMOMETER_____

CORRECTION FACTOR_____ DATE_____

CERTIFIED THERMOMETER #_____

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